Sudden large samples: opportunities and problems

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SUMMARY

Oilspills and other causes of mass mortalities in birds offer opportunities to study origins, biometrics, condition, pathologies and mortality impacts in populations, using statistically adequate samples; they also create problems of storage, availability of facilities and staff, funding and, most critically, labelling and cross-referencing. Experience at the National Museums of Scotland over 25 years, involving in particular divers, gulls, auks and buzzards, shows that the first crucial step is to buy time—although an inevitable expense—by freezing at least 20 specimens of each sex. If full study skins are too costly (or beyond the expertise of available staff) to prepare, preservation of the skeleton, one wing and sometimes the tail maximises opportunities for age, moult and other studies; but retaining the 'best' specimens may create biases. Preservation of muscle samples for later extraction of DNA is highly recommended. Publication of results should not be neglected, irrespective of time-lag since accession. Recommendations for procedures when processing oilspill samples are appended.

Introduction

Although the world's museums are filled with millions of bird skins, skeletons, spirit specimens and eggs, it is often difficult to find large enough samples to allow for comparisons between closely related species, subspecies or populations within species. Therefore, at a taxonomic level it can be almost impossible to determine whether apparent discrete geographical variation is real or an artefact of small, localised samples (Corbet 1970). It is also rarely possible to obtain information from existing collections on pathology, mortality or other aspects of population biology, which can be used to assist studies related to the management and conservation of species today and in the future.

In recent years at the National Museums of Scotland (NMS) we have had opportunities to study large samples of dead birds, many of which were subsequently preserved in a variety of ways for future reference. Most of these birds were either casualties of oilspills at sea or from natural 'wrecks' caused by extreme winter weather at sea. We are aware that other museums have been involved in analysing oiled dead seabirds, although few have placed an emphasis on specimen preparation as at NMS. In other cases samples have been collected over a few years by other institutions for specific studies and then passed on to NMS for our own use, or they have been the result of legal culls. In order to maximise the potential of these opportunistic samples, we have collaborated widely with other institutions and organisations, and consequently we have made samples available for a wide range of associated studies. From these large samples of birds we have carried out studies on age, sex, geographical variation/origins, hybridisation, pathology, diet, mortality, moult, etc., in collaboration with colleagues from the Universities of Edinburgh, Glasgow and

Liverpool John Moores, and the Institute of Terrestrial Ecology (now the Centre for Ecology and Hydrology).

Although large samples create opportunities, they also create huge problems in terms of storage of carcasses until needed, the availability of suitable facilities and casual staff to process the birds, sufficient funding to cover staff costs and materials and, most critically, the labelling and cross-referencing of multiple samples. We cannot claim to have solved all of these problems, but we offer our experiences to aid and warn others who might wish to embark on similar projects. In order to show the kinds of information that can be obtained collaboratively, we also briefly review the results of the various studies that have been carried out at NMS over the years on large samples of birds that have been acquired opportunistically and often suddenly.

Opportunities and problems

Large samples of birds often offer unique opportunities to look at populations in detail, which would not be possible under normal circumstances. Oilspills, in particular, cause mortality on a scale which would be considered unethical to cause intentionally for research purposes. By working with collaborators, it is possible to maximise the research potential of these samples. We have worked closely with veterinary pathologists from the University of Edinburgh to provide a greater insight into the effects of oil on seabirds and the frequencies of various natural diseases that afflict bird populations. By combining basic information on age, sex, moult and condition with pathological observations, we have been able to look at how pathology and mortality affect different segments of populations. We have also supplied fresh tissue samples for analyses of the concentrations of organochlorines and heavy metals, and for molecular studies.

In dealing with large samples, there are a number of logistical problems to overcome. Assuming that others have organised the collection of, for example, oiled seabirds and frozen them for future analysis, there are still the problems of finding sufficient funding to carry out a full analysis, suitable facilities where the preliminary processing of large numbers of birds can be carried out, and sufficient knowledgeable casual staff to work through the birds and prepare them. We were fortunate that in dealing with the dead seabirds from the *Braer* oilspill at Garths Ness, Shetland, in January 1993, Scottish Natural Heritage made a small grant available to finance the employment of a small number of experienced people for the preparation of voucher specimens. Local companies also gave materials generously for use at very short notice. In the case of the *Sea Empress* oilspill near Milford Haven in February 1996 we were contracted by the Countryside Council for Wales to work on the dead oiled seabirds, excluding the Black Scoters *Melanitta nigra*. However, in both cases the level of funding was less than ideal, and NMS covered much of the cost of recording and analysing data, preparing specimens and writing reports from its own resources. Our other studies on large samples (e.g. Common Buzzards *Buteo buteo* and Northern Fulmars *Fulmarus glacialis*) are being carried out over longer periods of time as staff and volunteer time become available. In all cases it has been absolutely essential

that all the material is frozen, so that time can be taken to plan the work ahead and contact potential collaborators, who might need fresh tissue samples. Also, smaller batches can then be thawed out to be worked on, thereby preventing deterioration of the whole sample. This systematic approach ensures the maximum research benefit in terms of multiple uses of each specimen.

Suitable facilities for the sorting, measuring and sampling of birds are difficult to find. By collaborating with the Royal (Dick) School of Veterinary Studies, we were able to use their excellent pathology laboratories which have ample space for several people and good facilities for storage of specimens, washing down benches, etc., and disposal of unwanted material. In recent years we have used our own (similar but smaller) specimen processing and maceration facilities at our West Granton Research Centre.

We have found casual staff through a network of interested ornithologists, otherwise unemployed, and some students. Ideally, processing is delayed until student holiday periods, so that an abundance of willing workers can be found. This requires freezing of the carcasses for several months, which may be difficult. We have used commercial cold stores for this, which creates additional expense. We should also caution that disposal of oily/detergent water from cleaning specimens should be done by a responsible specialist contractor, so that other fauna are not affected by liquid waste that would otherwise have been disposed of down drains. Again this can create an additional and significant cost.

One of the key factors in the analysis of large samples is to preserve statistically adequate samples of skins and/or skeletons for later research; a minimum of 20 adults of each sex is essential, but many more would be desirable. However, resource constraints often severely limit how many specimens can be prepared. These samples provide good data on the extent of shrinkage of skin measurements, which allow for comparison between other published studies of living birds. In checking preserved samples, we also found that inevitably a few mistakes had been made in the fresh measurements, but voucher specimens ensured that these mistakes could be corrected. Moreover, in the heat of the moment it is easy to miss the significance of certain characters or impossible to take additional measurements or record moult, etc., especially if birds are badly oiled.

The preparation of full study skins can be very time-consuming and is a specialist skill. Therefore, particularly in recent years, we have preserved many specimens as skeletons, but have retained one complete wing for ageing, moult and measurements. For some species, complete tails were also preserved, where these aided ageing (e.g. Common Buzzard). The preparation of this type of specimen requires relatively unskilled staff and is much quicker than the production of conventional study skins. The total number of specimens for large samples prepared since 1978 (and mostly since 1993) is shown in Table 1.

One of the biggest problems after completion of studies like the ones described below is finding sufficient time to prepare papers for publication. We have been moderately successful at this (see References), but we are all too aware that many

TABLE 1
Numbers of specimens of bird species prepared by NMS from oilspills and other sources since 1978.

Species	*Esso Bernicia* 1978/79	*Braer* 1993	*Sea Empress* 1996	Other sources 1978–1998
Gavia stellata			20	
Gavia immer	68	11	*2*	
Podiceps cristatus				25
Fulmarus glacialis		16		40
Phalacrocorax aristotelis		71		
Somateria mollissima		68		
Clangula hyemalis		108		
Melanitta nigra			18	
Oxyura jamaicensis				47
Buteo buteo				180+
Larus argentatus		12		
Larus fuscus				25
Larus marinus		20		
Larus glaucoides		13		
Rissa tridactyla		90		100+
Alle alle		13		
Alca torda	5	4	20	
Uria aalge	51	7	114	
Cepphus grylle	119	126		

data still need to be analysed and several papers await completion, e.g. on Red-throated Divers *Gavia stellata* and Black Guillemots *Cepphus grylle*. It is vitally important to not only preserve specimens for future research use, but also to publish the results in the scientific literature.

Finally, the success of projects like the ones described in this paper often rests on the enthusiasm and determination of particular individuals. We have been most fortunate that the late Douglas Weir dedicated a great deal of his time, often for very poor remuneration, to driving forward our efforts on many of the projects described below. His considerable ornithological expertise and his fearlessness in the face of large piles of dead birds were essential to our success in recent years.

Oiled seabirds

NMS has been involved in studies on seabirds from three oilspills, the *Esso Bernicia* in 1979, the *Braer* in 1993 and the *Sea Empress* in 1996. The latter two spills involved the Pathology Department of the Royal (Dick) School of Veterinary Studies in pathological examinations of large series of seabirds. Several other collaborators were supplied with samples at the time or subsequently. The separate species accounts below summarise briefly the significant results of a wide variety of investigations.

Divers Gavia

NMS has prepared nearly 100 Great Northern Divers *Gavia immer*, mostly from the *Esso Bernicia* (1978–1979 at Sullom Voe) and *Braer* (1993) oilspills in Shetland. Wing measurements indicated origins of the *Esso Bernicia* oilspill birds by comparison with measurements from known breeding birds. Wintering Great Northern Divers off Shetland consisted of approximately 45% birds from Iceland, 45% from Greenland and 10% from Canada (Heubeck *et al.* 1993, Weir *et al.* 1996a). From the estimated total number of Great Northern Divers that died in this spill, it was estimated that 10% of breeding females from Iceland were killed, which represents a serious level of mortality in this long-lived, slow-breeding bird (Weir *et al.* 1996a).

We radiographed many carcasses in 1980 to detect lead shot, but in particular noted from skeletons from the *Braer* oilspill that many had non-fatal gunshot wounds, i.e. holes that were healing that had been created by bullets or shot. Because the carcasses of many of the *Esso Bernicia* birds had been retained, it was also possible to prepare their skeletons and check for similar damage. About 34% of birds had non-fatal gunshot wounds and, because we knew of the birds' origins, it was possible to observe some regional differences in the use of ammunition. The skeletons of birds that had probably originated in Canada invariably had holes made by .22 rifle bullets, but Icelandic birds had been damaged by shotgun pellets.

Auks Alcidae

The origins of about 200 Guillemots *Uria aalge* and Razorbills *Alca torda* were investigated from the *Sea Empress* oilspill. Wing and culmen measurements revealed that these were from local breeding populations. It was estimated that mortality would be insignificant (<5%), as was shown in the subsequent breeding season (Weir *et al.* 1997).

Gulls Laridae

About 50 Kittiwakes *Rissa tridactyla* killed by the *Braer* spill were determined as of high-latitude origin from wing measurements (Weir *et al.* 1996b) and hence their mortality had no impact on breeding populations in Shetland. This large sample of winter birds provided valuable new information on the moult cycle of the Kittiwake (J. Conner pers. comm.), and allowed the testing of a morphometric method for determining sex (McGowan & Zonfrillo 1995).

The *Braer* spill coincided with an apparent invasion of Iceland Gulls *Larus glaucoides*. Most were nominate *glaucoides*, but two were of the form *kumlieni*, which had varying amounts of wing-tip melanism. These observations inspired a review of Iceland Gull records in Britain (Weir *et al.* 1995), showing an apparent increase in the proportion of *kumlieni* in invasions since the nineteenth century. We also recently completed a taxonomic review of Iceland Gulls which demonstrates that *L. g. glaucoides* has been replaced by *L. g. thayeri* in the Canadian Arctic and

that a variable hybrid (called *kumlieni*) appears to be spreading eastwards from Baffin Island (Weir *et al.* 2000).

Wrecks

Wrecks of dead seabirds occur regularly during the winter, although only in a minority of cases are the corpses utilised to their fullest potential. It would be valuable to study these in detail to see how in composition, pathology, etc., they differ from birds killed by oilspills. This could give a greater insight into which segments of populations of different species are vulnerable to these major causes of mortality. In 1997 a sample of 40 Northern Fulmars was collected from a localised wreck on the Northumberland coast. This large sample is currently the subject of a study of biometrics, origins and diet.

A sample of more than 130 wrecked Kittiwakes has also been collected recently from the Northumberland coast, which will complement origin, moult and other studies carried out on the *Braer* oilspill sample.

Samples collected by others

Common Buzzard Buteo buteo

We obtained more than 180 Common Buzzards from the Royal Society for the Protection of Birds (RSPB) and the Scottish Agricultural Science Agency (SASA). These had been collected over a number of years before being passed to us, thereby providing another opportunity for a combined morphometric and pathological approach. Scottish Natural Heritage kindly provided a small grant, which allowed the bulk of this study to be completed.

Preliminary findings have been published on a smaller sample of more than 60 birds, relating pathological findings to age and sex (Redrobe *et al.* 1997), and a paper on the full sample is currently in preparation. Most birds (55%) died from trauma or collision and starvation (23%). Poisoning was confirmed in 5% of birds (75% of those tested), although only suspicious deaths were investigated owing to the high costs of analysis. Gunshot wounds, not necessarily fatal, were present in 13% of birds. Most birds (73%) were subadults and most (74%) were male. This was thought to reflect the male offspring being driven out of parental territories and males participating more than females in aggressive territorial behaviour (for which see Weir & Picozzi 1975). These males have to survive in suboptimal habitats and consequently have a higher risk of death through, for example, foraging for carrion at roadsides. Diet was mostly small mammals (40%) and insects (17%). Parasites were recorded in 16% of birds, including only the second reported case in the species of the nematode *Cyathostoma*, which occurs in the orbital cavity.

Ruddy Duck Oxyura jamaicensis

Forty-seven cabinet skins were prepared from recently culled specimens of this duck from the British population. We feel it is important that a statistically adequate sample

is taken for this introduced species, in order to determine any morphological changes that have occurred through local adaptation and also as a record of the species, if it is eventually exterminated.

Conclusion

Large samples provide excellent opportunities for gathering large amounts of data for a wide variety of studies. However, logistically and in terms of resources needed, they can be very difficult to carry out. We have been fortunate in that we do have facilities for specimen preparation and we have found enthusiastic collaborators, small grants and sponsorship-in-kind to assist our efforts. The reward has been to initiate many different research projects, which have made maximum use of each specimen, and which have resulted in statistically adequate samples of data-rich skins, skeletons and wings for our collections.

There is potentially plenty of material out there. What we all lack are the resources to access the abundance of data that could benefit environmental and conservation biology. We would like to see a contingency fund established by the oil companies to provide immediate support for detailed investigations like those described above. Ideally all available specimens would be preserved; we are concerned that preservation of the 'best' specimens may be biasing samples and unduly influencing our observations. These sudden large samples provide excellent opportunities for collaboration between museums and other research institutions, in order to maximise the potential for research and preservation of specimens. We would welcome further discussion and dialogue with our museum colleagues about how this can best be achieved.

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Appendix

Recommendations for the processing of dead bird specimens from oilspills

Plan the processing of the bird specimens well. It saves much time and effort, and ensures that data are not inadvertently lost. Ensure that all procedures and processes requiring COSSH* and risk assessments are considered prior to starting work. Ensure that all temporary staff are fully briefed, trained and have read the appropriate COSSH and risk assessments prior to starting work.

1. *Recording data*: Standard data sheets are recommended, in order to ensure that all data are recorded. We found that it was more effective having one person writing down the data recorded from one or more persons, because of the considerable mess caused by the oil. This limited contamination of data sheets by oil and facilitated the detection of anomalous data or measuring errors.

2. *Labelling*: It is essential that good labelling is used to prevent loss of data from specimens, especially while going through a variety of processes. We have used embossing tape (e.g. Dymo), aluminium tape or tags imprinted with numbered metal punches, and plastic plant tags with indelible ink. The latter are least successful as the ink may not quite survive the skeletonisation process. All parts of a specimen must be labelled, e.g. wing, skeleton, skin, etc.

3. *Samples*: The preservation of a muscle sample (e.g. pectoral muscle) for later extraction of DNA is highly recommended. NMS stores these deep frozen at "-40°C, although "-70 °C is recommended for long-term storage. Alternatively, thin slices of muscle can be preserved in 75% ethanol (not IMS [industrial methylated spirit] or formalin) at room temperature. Other kinds of samples may be required for other studies (e.g. for PCBs, heavy metals etc.). Have plenty of self-sealing sample bags available, on which informaton can be written. It is often useful to place labels in the bags in case the writing becomes obscured. It is vital to ensure that all samples are correctly labelled for later cross-referencing with other data and specimens.

4. *Measuring*: We recommend using plastic dial calipers for bill and tarsus measurements, as these can be cleaned more easily. As sand and oil are damaging to the calipers, they should be regulary checked for accuracy. Also, they are relatively low cost if they have to be disposed of. For wing measurements we recommend standard end-stopped rules (or similar) set into larger measuring boards. To ensure consistency, one individual was generally responsible for measuring.

5. *Protective clothing*: We have used paper boiler suits with disposable polythene aprons and huge numbers of latex gloves. Wellington boots are recommended as footwear.

6. *Sorting*: Although it is preferable to sample all specimens, this may not be practical owing to very large numbers, limited time and high cost. Inevitably, most effort is concentrated on specimens in good condition (even if heavily oiled), so that age and sex are most likely to be recordable. Badly decaying specimens may not be sexable and may pre-date the oilspill. Be aware that selecting the birds in best condition may inadvertently introduce a sampling bias, especially if males and females of a species arrive at a location at different times, or if body size and/or condition affect rate of decay.

7. *Measurements and external examination*: We record appropriate standard measurements from wing, tail, tarsus and bill. It is often not practical to measure body mass, because of oil, sand and water-logged plumage. Colouration of the irides and soft parts (bill, face and legs) are recorded if possible and appropriate. Relevant plumage characters are noted for ageing, etc.

8. *Internal examination*: A selected sample of the birds should be examined by an experienced avian pathologist. However, there may be some conditions that can be identified by most recorders with some training, e.g. aspergillosis. Crop contents are stored in 70% ethanol or deep frozen for later examination. Measurements of gonads (using dial calipers) involve length and width of testes and diameter of the largest ovarian follicle. We have attempted to get recorders to measure the bursa of Fabricius, but this was often difficult owing to heavy oil contamination and/or internal trauma caused by carcasses being crushed at the bottom of heavy storage sacks. The preparation of skeletons allows for later examination of skull ossification, but birds prepared for skins should have their skull ossifcation recorded at the time of preparation. Subcutaneous and internal fat deposits are recorded subjectively on a four-point scale from 0 to 3. As birds begin to decay rapidly once thawed, it is important not to thaw out too many birds for a daily examination otherwise valuable data may be lost.

9. *Removing the oil from specimens*: We have used petrol, paraffin and powerful detergents to remove oil from plumage. Appropriate COSSH and risk assessments need to be carried out for all processes prior to working on the dead seabirds.

10. *Preparing skeletons*: We have used autoclave bags in order to keep skeletons separate during maceration at 60°C. We have generally preserved one wing from each skeletonised bird, so that wing length, moult and other plumage features can be recorded. This allows for later examination and also for the measurement of wing shrinkage to facilitate a suitable correction factor.

11. *Clearing up*: All animal waste should be bagged in appropriate polythene sacks, labelled with a description of the contents and the address of the institution they have come from, before being incinerated. Note that plastic waste and latex gloves may have to be incinerated separately and should be bagged and labelled separately. Waste oily water should be left to settle in plastic drums, so that the oil can be decanted off for disposal by a specialist company.

*Control of Substances Hazardous to Health